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CONTINUOUS EXPOSURE OF ANIMALS TO  
METHYLISOBUTYLKETONE

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Aerospace Medical Research Laboratory  
Wright-Patterson Air Force Base, Ohio

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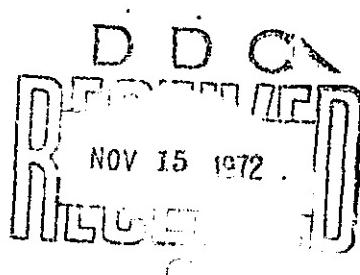
## 13. ABSTRACT

This report was presented at the Proceedings of the 2nd Annual Conference on Environmental Toxicology, sponsored by the SysteMed Corporation and held in Fairborn, Chio on 31 August, 1 and 2 September 1971. Major technical areas discussed included toxicological evaluation of volatile halogenated compounds, protection of the public against air pollution and toxicological problems with aircraft, missiles, and space vehicles.

## Key words:

- Continuous exposure
- Pathology
- Toxicological screening
- Gas chromatography
- Electron microscopy
- Propellant toxicity

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METHYLISOBUTYLKETONE

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INTRODUCTION

Methylisobutylketone (MIBK), also known as isopropylacetone, is a common solvent and vehicle for lacquers, oils, fats, waxes, gums, and resins. Its excellent solvent properties make it useful in fire resistant plastic materials currently under test by NASA for possible use in space cabins. The manufactured products contain, entrapped in the plastic, some residual MIBK which will outgas under reduced pressure conditions and may appear as a contaminant in the spacecraft environment.

Methylisobutylketone has a relatively low order of acute toxicity with mice surviving 30-minute exposure to 19,500 ppm (Shell Chemical Corporation, 1957), rats surviving 4-hour exposure to 2,000 ppm (Smyth, 1956), and guinea pigs surviving after exposure to 1,000 ppm for 6 hours (Specht, 1938).

The American Conference of Government Industrial Hygienists (1970) has recommended a threshold limit value (TLV) of 100 ppm for this compound.

Industrial experience with MIBK has not shown any adverse physiological effects on man other than headache or nausea at or around the TLV of 100 ppm. Elkins (1959) reported that exposed workers developed some tolerance to MIBK during the working week but lost this tolerance over the weekend. Silverman et al. (1946) found that a 100-ppm exposure to MIBK was acceptable to 12 human volunteers for a 15-minute period, but that 200 ppm was objectionable due to odor intensity. Because high air concentrations of MIBK have a narcotic action which would affect human

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performance, further information about prolonged or continuous exposure to this chemical was desired. A 90-day continuous exposure was selected as being best able to determine toxicological effects under space cabin conditions. In order to determine the MIBK concentrations to be used in this exposure, two-week range-finding experiments were conducted at 820 and 410 mg/m<sup>3</sup> MIBK under ambient conditions. Based on the results of the range-finding exposures, a concentration of 410 mg/m<sup>3</sup>, equivalent to 100 ppm at ambient pressure, was chosen as the MIBK concentration in the 90-day experiment.

## METHODS

Animal exposure facilities (MacEwen, 1965; Thomas, 1968) of the Aerospace Medical Research Laboratory were used for both the two-week and the 90-day continuous experiments. Atmosphere flow was maintained at 40 cfm and chamber temperature at 72 F in both exposures. The ambient experiments were carried out in air, and the pressure maintained at 725 torr to seal the chamber and prevent contamination of the surrounding laboratory environment with MIBK vapor. The 90-day study was performed at 260 torr using a 68% O<sub>2</sub> - 32% N<sub>2</sub> atmosphere.

Liquid MIBK, highest purity, was introduced into an all glass vaporizing unit by means of a dual syringe pump from a large reservoir. Dry air flowing through the heated vaporizer carried the MIBK vapor through a flowmeter and metering valve system into the chamber air supply duct. The stainless steel tubing between the vaporizer and metering valve was heated to prevent recondensation of the MIBK. Heating was not necessary after dilution in the chamber air supply duct.

A gas chromatographic procedure was developed for contaminant monitoring on a semi-continuous basis. Air samples were taken from a position in the chamber just above the breathing zone of the dogs and continuously pumped to the analyzer system where an automatic sampling valve took samples every five minutes. The samples were introduced directly into the gas chromatograph sample inlet.

The MIBK in the gas sample was separated on a 10-inch column of Porapak Q operated at 190 C and detected with a flame ionization detector. The retention time of MIBK in this system was 1.5 minutes, which allowed convenient sampling at five-minute intervals. MIBK vapor calibration standards made up in Mylar® bags were used daily and a variation in detector response of  $\pm$  5% was found. The variation from one bag to another when run the same day was approximately 2%.

## RESULTS AND DISCUSSION

### Range-Finding Experiments

Test animals for each exposure included four rhesus monkeys, eight beagle dogs, 40 ICR mice, and 50 Wistar rats. As controls, three monkeys, four dogs, 20 mice,

and 25 rats were placed in another Thomas Dome under the same conditions, with the exception of contaminant. One monkey in each group had cortical electrodes implanted for evaluation of CNS effects.

Test programs were designed to evaluate the inhalation effects of the MIBK exposure as shown in table I.

TABLE I  
TESTS FOR DETERMINATION OF MIBK EFFECTS

Preexposure Tests

Body Weight - monkeys, dogs, rats  
Clinical Serum Chemistry - monkeys, dogs  
Hematology - monkeys, dogs  
EEG - monkeys

During Exposure Tests

Spontaneous Activity Measurement - dogs  
Symptomatology - all animals  
Mortality Response - all animals

Postexposure Tests

Body Weight - monkeys, dogs, rats  
Organ to Body Weight Ratios - rats  
EEG - monkeys  
Clinical Serum Chemistry - monkeys, dogs  
Hematology - monkeys, dogs  
Pathology - all animals  
Blood pH and Gases - dogs

Table II details the individual tests performed in the hematological and clinical serum chemistry examinations.

TABLE II  
HEMATOLOGY AND CLINICAL SERUM CHEMISTRY TESTS  
PERFORMED TO DETERMINE MIBK EFFECTS

<u>Hematology</u>	<u>Serum (continued)</u>
Hematocrit	Calcium
Hemoglobin	Total Phosphorus
Red Blood Cell Count	Total Bilirubin
White Blood Cell Count	Albumin
<u>Serum</u>	Total Protein
Sodium	Uric Acid
Potassium	Blood Urea Nitrogen
Cholesterol	Glucose
	Alkaline Phosphatase
	Creatinine
	Chloride

There were no signs of toxic response during exposure to 820 mg/m<sup>3</sup>. At the end of the two-week exposure period, there was no difference in cortical activity between the exposed and control monkeys nor were any significant differences observed in hematologic or clinical serum chemistry measurements for either dogs or monkeys. Gross pathologic examination of tissues from both exposed and control animals failed to reveal any apparent differences except for the case of rat kidneys, which appeared slightly mottled. Blood gas measurements made on dogs did not show any effects attributable to MIBK exposure.

Organ weight and organ to body weight ratios were evaluated and the kidneys and livers were found to be significantly heavier in the rats exposed to MIBK, as shown in table III.

The animals exposed to 410 mg/m<sup>3</sup> MIBK showed no outward toxic effects that could be attributed to the two-week exposure. Again, the only effect observed was on rats in which kidneys were significantly enlarged when compared to those in the control group. This is demonstrated in table IV.

From the data obtained in the range-finding experiments, the kidney appeared to be the organ primarily affected by exposure to MIBK. Histopathological examination of rat kidneys revealed some changes which are discussed by Col. MacKenzie in his presentation at this conference (MacKenzie, 1971).

TABLE III

EFFECT OF TWO-WEEK EXPOSURE TO 820 mg/m<sup>3</sup> MIBK  
ON ORGAN WEIGHTS OF ALBINO RATS

	Mean Organ Weight (grams)	Mean Organ/Body Weight Ratio (grams/100 grams body weight)	
	<u>Test</u>	<u>Control</u>	
	N = 50	N = 50	N = 50
Heart	0.9	0.9	0.357*
Lung	1.3	1.3	0.499
Liver	9.0**	8.2	3.445**
Spleen	0.8	0.8	0.291
Kidney	1.8**	1.5	0.694**

\*Different from control mean at the 0.05 significance level.

\*\*Different from control mean at the 0.01 significance level.

TABLE IV

EFFECT OF TWO-WEEK AMBIENT EXPOSURE TO 410 mg/m<sup>3</sup> MIBK  
ON ORGAN WEIGHTS OF ALBINO RATS

	Mean Organ Weight (grams)	Mean Organ/Body Weight Ratio (grams/100 grams body weight)	
	<u>Test</u>	<u>Control</u>	
	N = 50	N = 25	N = 50
Heart	1.0	0.9	0.416
Lung	1.2	1.3	0.547
Liver	8.6	8.4	3.756
Spleen	0.8	0.8	0.353
Kidney	1.7*	1.5	0.729*

\*Different from control mean at the 0.01 significance level.

90-Day Continuous Exposure

Based on the range-finding results, the 410 mg/m<sup>3</sup> MIBK exposure level was selected for the continuous 90-day study under simulated space cabin conditions.

The animal species selected for exposure to MIBK for 90 days were: 100 albino rats (Wistar strain); 8 beagle dogs; 2 rhesus monkeys.

As noted previously, test animals were exposed to 410 mg/m<sup>3</sup> MIBK vapor for a period of 90 days in an altitude chamber operated at 260 torr pressure using a 68% O<sub>2</sub>-32% N<sub>2</sub> atmosphere. The control group of animals was maintained in a separate altitude chamber under identical environmental conditions, except that no MIBK was present.

All dogs were examined biweekly, including the month prior to initiation of the experiment. At the time of each examination, the dogs were weighed and blood samples were taken for hematology and the battery of clinical serum chemistry tests previously detailed in table II.

Liver function tests (bromsulphalein [BSP] dye retention) were performed preexposure and immediately postexposure. Serum acid phosphatase and serum glucuronidase determinations were done preexposure and at 30 and 60 days.

At termination, two dogs from each group were transferred to the postexposure holding room for 60 days to determine reversibility of effects should any lesions be found. The remaining six dogs in each group were sacrificed, examined grossly, and samples of liver, brain, kidney, heart, lung, spleen, and endocrine glands were taken for histological evaluation.

Rats were weighed preexposure and biweekly during the exposure period to determine growth rate. Two rats from each group were necropsied at weekly intervals for three weeks and then at biweekly intervals thereafter. After two weeks of exposure, 10 rats were removed from each chamber and necropsied in groups of two at biweekly intervals to determine reversibility of the kidney lesions seen in the preliminary experiments. At termination of the experiment, 10 rats from each group were removed and saved for serial sacrifice for reversibility studies, 10 were submitted to histopathology, and the remaining rats were necropsied and the visceral organs weighed for determination of organ to body weight ratios.

Clinical serum chemistry, hematology, enzyme, and bromsulphalein tests on dogs and monkeys did not reveal any biologically significant differences between the exposed animals and their controls.

The growth rate (measured biweekly) of both the exposed and control groups of rats are shown in figure 1. There was no effect upon the growth rate as a result of continuous exposure to MIBK for 90 days.

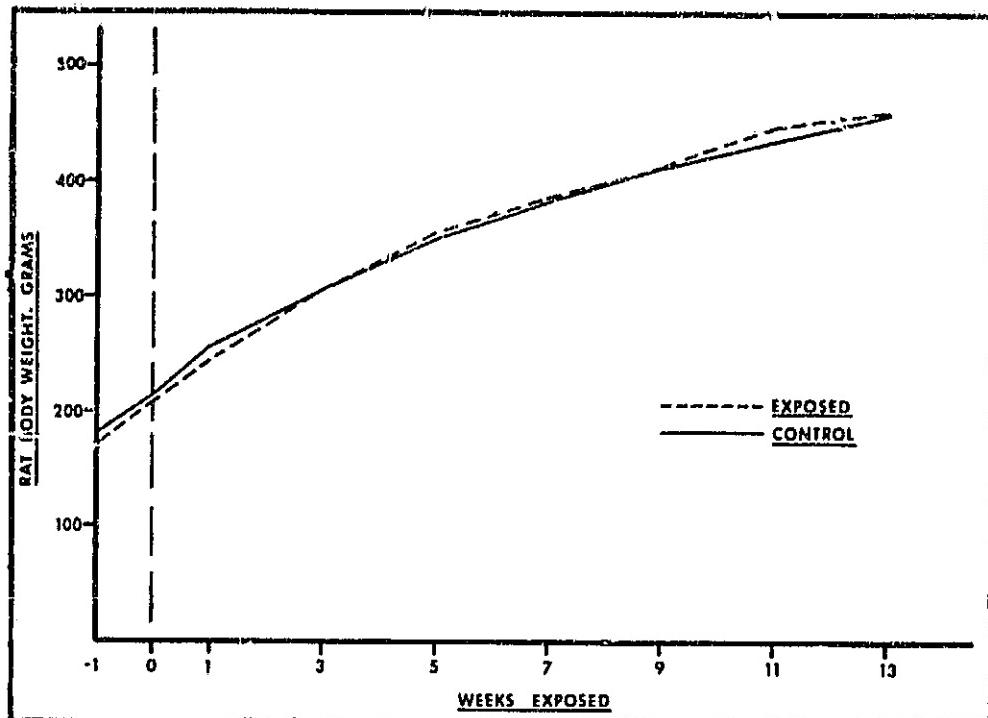


Figure 1. MEAN GROWTH RATE OF RATS EXPOSED CONTINUOUSLY TO 410 mg/m<sup>3</sup> MIBK AND CONTROLS.

The effect of 90-day exposure to MIBK on albino rat organ weights and organ to body weight ratios is shown in table V. There is a statistically significant difference between exposed and control liver and kidney weight means with a corresponding increase in organ to body weight ratios for the samples from exposed animals.

Some significant histopathological changes were seen in the kidneys of exposed rats and these are discussed by Col. MacKenzie in his review of the pathological effects of MIBK exposures.

TABLE V

EFFECT OF 90-DAY ALTITUDE EXPOSURE TO 410 mg/m<sup>3</sup> MIBK  
ON ORGAN WEIGHTS OF ALBINO RATS

	Mean Organ Weight (grams)	Mean Organ/Body Weight Ratio (grams/100 grams body weight)	
	<u>Test</u>	<u>Control</u>	
	N = 56	N = 55	N = 56
Heart	1.3	1.3	0.302
Lung	1.5	1.5	0.352
Liver	10.8*	9.9	2.477*
Spleen	0.7	0.7	0.159
Kidney	3.1*	2.6	0.713*

\*Different from control mean at the 0.01 significance level.

## SUMMARY

Continuous exposure of dogs, monkeys, mice, and rats to MIBK for two weeks and all animals except mice for 90 days resulted in measurable adverse effects only in the case of rats. Rat kidney weights and kidney to body weight ratios were significantly elevated after exposure to 410 mg/m<sup>3</sup> for two weeks, and kidney and liver organ weights and organ to body weight ratios were elevated after exposure to 820 mg/m<sup>3</sup> for two weeks and to 410 mg/m<sup>3</sup> for 90 days.

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## DISCUSSION

FLOOR: This was not at altitude, was it?

MR. VERNOT (SystemMed Corporation): The range-finding experiments were not at altitude, but the 90-day experiment was at altitude and under space cabin conditions - corrected oxygen, etc.

DR. THOMAS (Aerospace Medical Research Laboratory): I want to point out again that the Air Force is not in the man-in-space business. This study was sponsored and funded by NASA. And the reason why I'm so particular is that last year I lost almost \$200,000 due to the fact that some Congressman picking up our program documentation said, "What the hell is Anton doing in man-in-space?" So when you come here and hear about man-in-space, please keep in mind that all these studies are sponsored by NASA, and the Air Force is not doing these on its own.

MR. VERNOT: That is certainly true. It is also true that they have to come to Dr. Thomas' facility because there is no other place in the world that they can get this kind of work if they want it done.

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